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TITLE: Determination of the Role of Estrogen Receptors and Estrogen Regulated Genes in B Cell Auotreactivity

PRINCIPAL INVESTIGATOR: Betty Diamond

CONTRACTING ORGANIZATION:

The Feinstein Institute for Medical Research
Manhasset, NY 11030

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14. ABSTRACT

Systemic lupus erythematosus (SLE) is an autoimmune disease that occurs preferentially in women. In murine models of SLE, it is clear that increased or sustained high physiologic levels of estradiol can accelerate onset of disease and exacerbate disease severity. We have shown that estradiol alters B cell maturation in vivo but does so in a genetically restricted fashion. We have also shown that estradiol can act directly on B cells to alter B cell receptor (BCR) signalling strength.

This proposal is to understand which estrogen receptors mediate the effects of estradiol on B cell survival, maturation and activation in order to assess whether hormonal manipulation has a potential therapeutic role in SLE. The proposal is further designed to ask why estradiol affects B cell function in mice of one genetic background but not another.

15. SUBJECT TERMS
Systemic Lupus, Estrogen, BCR Signaling, B Cell Maturation, B Cell Selection

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Introduction:

Progress continues to be made in all aims of the proposal; one manuscript has been published in the past year, one manuscript is being revised as requested by the journal and one is being prepared for submission.

Body:

1) Determine which estrogen receptor is responsible for estrogen-induced alterations in BCR signaling.

As previously reported, we have studied mice lacking either estrogen receptor (ER) α or β to determine which ERα is responsible for the altered B cell maturation and the attenuated B cell receptor (BCR) signaling we observed in estradiol-treated mice. We found that engagement of either ERα or ERß led to a decrease in B cell lymphopoiesis and an expansion of marginal zone (MZ) B cells.

We also determined that ERα engagement alone caused an expansion and activation of autoreactive B cells. We confirmed these studies in wild type mice given ERα or ERβ specific agonists. These studies demonstrate that ERα is the dominant ER in the estradiol-induced alterations in B cell maturation and selection. The manuscript reporting these data is now in press in Molecular Medicine.

We believe these studies suggest that an ERα specific antagonist, delivered to B cells, might be an effective therapy for some patients with SLE.

2) Analyze B cell maturation and selection in placebo or estrogen-treated C57Bl/6 mice.

We previously observed that the estradiol-induced increase in titers of anti-DNA antibody in R4A transgenic mice is strain dependent. BALB/c mice are responsive to estradiol while C57B/6 mice are not. Both strains harbor the same anti-DNA heavy chain transgene with the same copy number and same chromosomal insertion site. As previously reported, expression of estrogen receptors and metabolism of estradiol did not differ between the strains. Estradiol altered B cell maturation in a similar fashion in both strains. B cell subsets in the spleen were also altered similarly by estradiol treatment in both strains. There was an estradiol-induced increase in transgene-expressing B cells in the spleens of both C57Bl/6 and BALB/c mice.

Estradiol rescued DNA-reactive B cells only in BALB/c mice. This reflects the fact that transitional B cells from BALB/c mice were less vulnerable to anti-IgM mediated apoptosis after estradiol exposure. The altered vulnerability to apoptosis was not seen in estradiol-exposed C57Bl/6 transitional B cells. The estradiol-induced attenuation of apoptosis was evident only in the transitional 2 (T2) B cell compartment. Similarly, the reduction in BCR-mediated calcium flux was present only in T2 cells. We now know this

reflects, in part, an estradiol-induced upregulation of the anti-apoptotic gene p202b in BALB/c transitional B cells but not C57Bl/6 B cells (Fig 1). This gene has previously been reported to reside in an SLE susceptibility locus. We are currently preparing a manuscript for submission.

3) Determine the role of antigen in estradiol-induced changes in B cell function.

R4A transgenic mice given estradiol display elevated serum titers of anti-DNA antibodies. When they are give both estradiol and DNase they do not develop autoimmunity. Heat inactivated (HI) DNase does not diminish the effect of estradiol; thus, the enzymatic activity of DNase is required. DNase does not reverse the expansion of transgene-expressing B cells, although studies of B cell repertoire show that it does reverse the estradiol-induced changes in B cell maturation and prevent the survival of high affinity DNA-reactive B cells (Table 1). We have also shown that DNase given after estradiol administration protects glomerul. This suggests that DNase removes available antigen thereby markedly reducing positive selection of autoreactive B cells. This has been confirmed in a study of glomerular binging by serum of mice treated with P or E2 and DNase showing that only serum of E2 an E2 plus H1 Dnase has high affinity glomerulotropic anti-DNA antibodies (Fig 2). The manuscript describing these data is being revised for publication.

Key Research Accomplishments:

1**.** We have demonstrated that the maturational changes in B cells induced by estradiol are mediated through ERα.

2. We have demonstrated that ERα engagement breaks B cell tolerance in a strain-specific fashion.

3. We have shown that p202b is upregulated by estradiol when B cell selection is altered.

4. We have shown that the effects of estradiol require the presence of antigen.

Reportable outcomes:

Invitation to AARDA 2011 symposium “Sex, Pregnancy, and Autoimmunity”, *Betty Diamond* – DATE: TBD – LOCATION: TBD.

Invited speaker at FoCIS Meeting “Selection of the B Cell Repertoire”, *Betty Diamond* – June 24-27, 2010 – Boston MA.

Invited speaker at SLE symposium “Setting thresholds for B cell selection: implications for autoimmune disease”, *Betty Diamond*- April, 2010 – Baylor Institute for Immunology Research, Dallas, TX

Hill, L., Jeganathan, V., Chinnasamy, P., Grimaldi C., and Diamond, B. Role of estrogen receptor α in B cell maturation and selection (in press in Molecular Medicine).

One manuscript in revision.

One manuscript in preparation.

Conclusion:

We have now clearly shown that ERα and ERß engagement can both lead to estrogen-induced alterations in B cell maturation but only ERα engagement interferes with negative selection of autoreactive B cells. It will, therefore, be important to test ERα antagonists in murine studies of B cell development and in murine models of lupus. This approach to therapy might provide clinical benefit without immunosuppression or intolerable masculinization in women.

The continued studies of estrogenic effects on B cells may identify other molecules that are critical in lupus pathogenesis and can be modulated to therapeutic benefit. These studies may have implications for many diseases with phenotypes that are altered by hormone exposure.

References:

Hill, L., Jeganathan, V., Chinnasamy, P., Grimaldi C., and Diamond, B. Role of estrogen receptor α in B cell maturation and selection (in press in Molecular Medicine).

Jeganathan, Venkatesh, Hajime Yoshifuji, Daisuke Kawabata, Prameladevi Chinnasamy, Anfisa Stanevsky, Christine M. Grimaldi, Joel Cohen-Solal and Betty Diamond. Antigen is required for Maturation and Activation of Pathogenic Anti-DNA Antibodies and Systemic Inflammation (under revision Journal Immunology).

Cohen-Solal, JFG., and Diamond, B. Strain specific effects of estradiol on B cell function (in preparation)

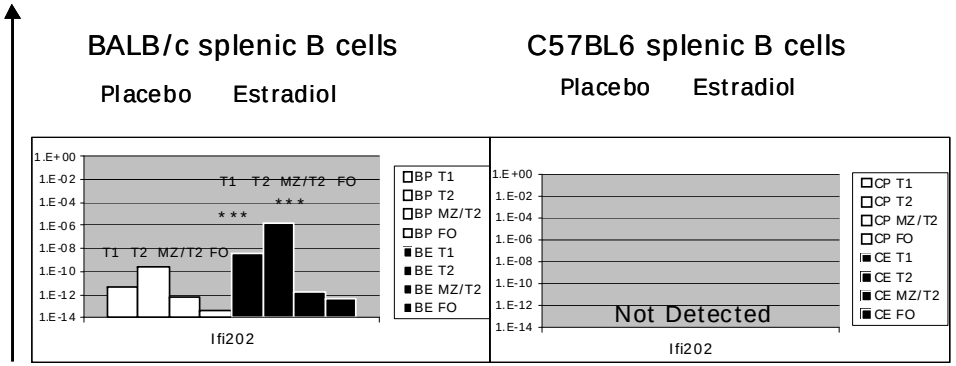
Appendices:

None

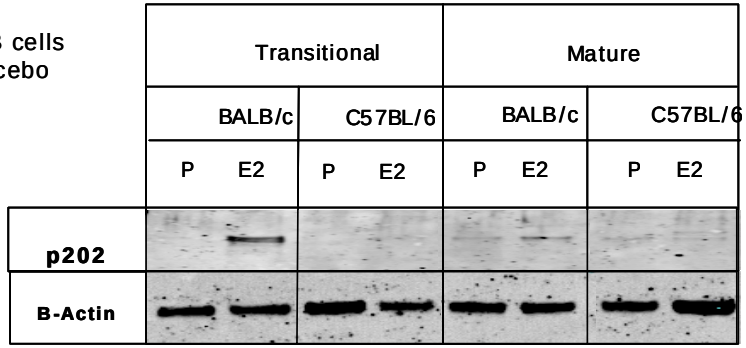
Supporting Data:

See attachments of Figures

a) In vivo expression of ifi202 by splenic B cells from mice treated with estradiol or Placebo for 4 weeks (qPCR)



b) In vivo expression of p202 by splenic B cells from mice treated with estradiol or Placebo for 4 weeks (western Blot)



c) In vitro induction of ifi202 expression by exposure to E2, IFNa or ICI 182-780 / 18h (qPCR)

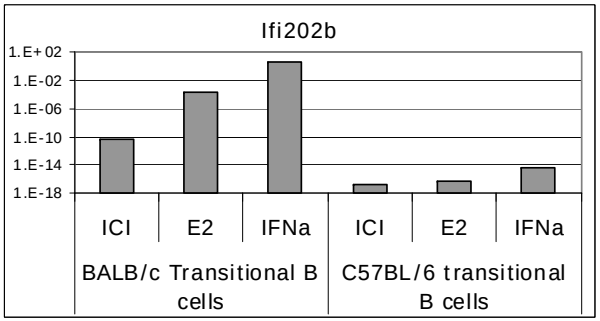


Figure 1 : Differential expression of p202 by transitional B cells from BALB/c and C57BL/6 mice treated with Estradiol in vivo or in vitro.

Figure 2.

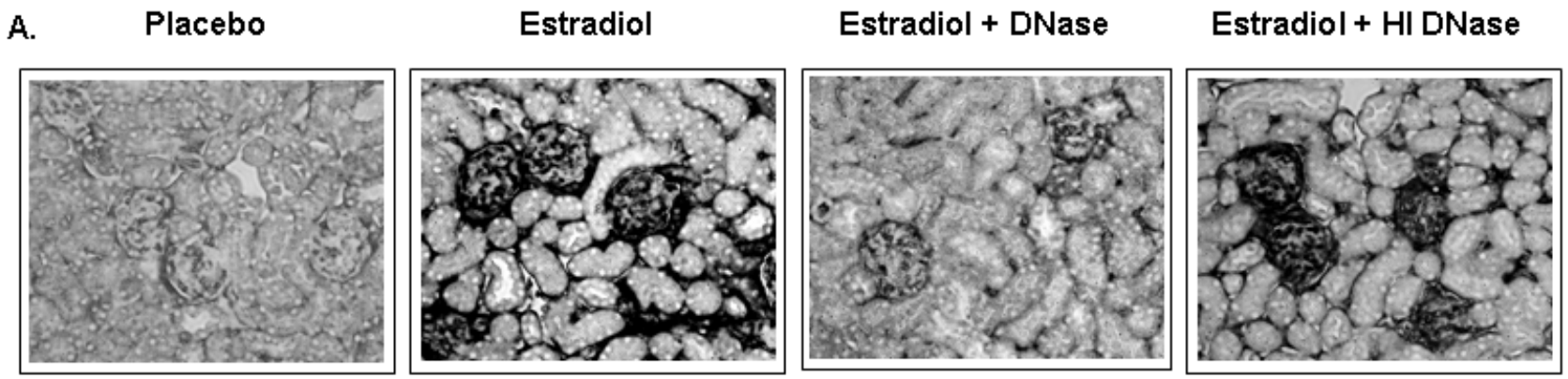

A.
Placebo
Estradiol
Estradiol + DNase
Estradiol + HI DNase
B.
No. of positive glomeruli
30
20
10
0
p<0.01
p<0.03
p<0.04
P
E2
E2+DNaseΘ
E2+HI DNaseΘ

Table 1. Frequency of high affinity DNA-reactive B cells in R4A Tg mice treated with E2 with or without DNase.

	Placebo E2		E2+DNase	E2+heat-inactived DNase
Transitional	6/50 (12%)	12/52 (23%)*	8/63 (12.6%)^{ns}	13/47 (29.7%)*
Mature	5/60 (8.3%)	18/62 (27.7%)*	8/72 (11.1%)^{ns}	13/57 (22.8%)*

A significant increase in high affinity anti-DNA B cells in R4A Tg mice treated with E2 was observed and was abrogated by treatment with DNase but not HI DNase. Fisher's exact test was used to analyze significance between the various treatment groups compared to the P-treated group. (*$p<0.05$), ns=not significant.